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INSECTICIDAL COMPOSITIONS

The present invention relates to plant essential oils and extracts and their uses as insecticides both in medical and non-medical applications. In particular the invention relates to the oils and extracts of Spanish sage (*Salvia lavandulifolia;* syn. *S. lavandulaefolia*) and *S. officinalis* "petite feuille Banon" and other essential oils or plant extracts such as those from *Artemisia dracunculus* (Tarragon), *Citrus limon* (Lemon), *Juniperus communis* (Juniper), *Laurus nobilis* (Bay), *Myristica fragrans* (Nutmeg), *Origanum vulgare* (Oregano), *Piper cubeba* (Cubebs), *Aloysia gratissima* (Whitebrush) and species of *Salvia* other than *S. lavandulifolia* which have the ability to kill a range of ectoparasites in a range of formulations.

Sucking lice (*Anuplura*) are a common pest of humans and animals which have a very wide global distribution, and are often spread by physical contact. In humans, lice are of two main genera. Lice of the genus *Pediculus* include head lice (*Pediculus humanus capitis* syn. *P. capitis*) and clothing lice (*P. humanus humanus* syn. *P. corporis*), whilst the commonest lice of the genus *Pthirius* are the crab (or public) lice, *Pthirius pubis*.

Head lice are a very common problem in children, from whom the insects can then be spread to other family members. Infestations are by no means limited solely to children. In the strictest sense, lice are obligate parasites, and hence are unlikely to leave a host voluntarily, yet whether infestations can be spread by inanimate objects, such as combs or hairbrushes remains a controversial issue. Louse

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infestations are usually accompanied by itching, although it is also possible that the insects could act as vectors for bacteria responsible for skin complaints, such as impetigo and scalp pyoderma (see Burgess (1995) *Advances in Parasitology* 36:271-342). In the UK, infestation rates were recorded as high as 23.1 % in primary school-aged children, and higher still (up to 30.3 %) in secondary schools. Other surveys suggest that one third of primary/junior school children are infested at least once per year (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO).

There is evidence that a recrudescence of clothing lice, which are generally rarer than head lice in "developed" countries, is occurring (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO). These insects have been linked to the transmission of trench fever, relapsing fever and typhus (see Van Der Lann and Smit (1996) *Nederlands Tijdschrift voor Geneeskunde* 140: 1912-1915).

Conventional treatment of head lice depends on application of insecticidal compositions and/or physical removal of lice and nits (lice eggs) by mechanical means (usually a fine-toothed comb). Insecticides used for treatment of head lice include organophosphates (e.g. malathion), pyrethroids, lindane and DDT. There are a number of problems associated with these compounds, including health concerns over exposure to organophosphates, development of resistance by lice (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO) and an undesirable product formulation (e.g. unpleasant smell). Some

preparations also contain very high (>80% v/v) concentrations of alcohol, which has been linked to instances of allergenic reactions.

A number of plant-derived treatments for lice have been developed previously, ranging from *Stemosa tuberosa* and *Hyssop officinalis* extracts, quassia chips, pyrethrins, rotenone (from *Derris* or *Lonchocarpus* spp.) to a number of essential oils. Essential oils which have been used, or suggested for use against lice, or which have been studied scientifically include *Pimpinella anisum* (Aniseed), *Cinnamomum zeylanicum* (Cinnamon), *Cymbopogon nardus* (Citronella), *Eucalyptus* spp. (Eucalyptus), *Pelargonium graveolens* (Geranium), *Hyssop officinalis* (Hyssop), *Juniperus communis* (Juniper), *Lavandula officinalis* (Lavender), *Citrus limon* (Lemon), *Cypressus* x *leylandii* (Leyland cypress), *Myrtus communis* (Myrtle), *Myristica fragrans* (Nutmeg), *Origanum vulgare* (Oregano), *Mentha* x *piperita* (Peppermint), *Pinus sylvestris* (Pine), *Thymus zygis* (Red thyme), *Rosemarinus officinalis* (Rosemary), *Melaleuca alternifolia* (Tea tree) and *Cananga odorata* (Ylang ylang). A number of these preparations have, however, been found to be of limited efficacy or have been associated with mammalian toxicity (see Burgess (1995). *Advances in Parasitology* 36:271-342). US patents 5,227,163 and 5,411,992 to Eini claim that a number of essential oils, including sage, and also a range of terpenoid compounds possess lice-repellent activity. Rosemary and Eucalyptus essential oils have similarly been demonstrated to possess repellent activity towards clothing lice (see Mumcuoglu *et al* (1996) *Entomologia Experimentalis et Applicata* 78: 309-314). Patent Application GB 2,341,091 also describes eucalyptus essential oil, in combination

with lavender and tea-tree essential oils, as a treatment for head lice. Combinations of essential oils, including rosemary, eucalyptus, tea-tree and lavender, as well as those from a number of other species, are also proposed as compositions suitable for lice treatment (WO00/00213).

Ectoparasites of livestock, such as sheep, can detrimentally affect productivity of milk and meat, and the quality of wool and leather. Additionally, the welfare of animals infested with parasites can be seriously affected (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). Conventional treatment and control regimes are expensive to implement, and can be associated with health risks to the farmer.

Sheep scab results from infestation by *Psoroptes ovis* (sheep scab mite), and is usually treated by plunge dipping in washes containing organophosphates (such as diazinon or propetamphos) or synthetic pyrethroids (flumethrin or cypermethrin). Organophosphate dips have the advantage that a single immersion is sufficient for treatment (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 46, Blackwell Science), but have been linked to health concerns for farmers (e.g. see Rees (1996). *Occupational and Environmental Medicine* 53: 258-263), whereas pyrethroid dips need to be used twice at 14 day intervals for effective treatment. *P. ovis* is usually restricted to sheep, although Psoroptic mange in cattle has been reported, and is a major problem in mainland Europe and the United States of America. The ear mite, *P. cuniculi*, is a close relative of *P. ovis*, and is primarily associated with rabbit populations, although it has also been isolated from sheep, goats

and horses.

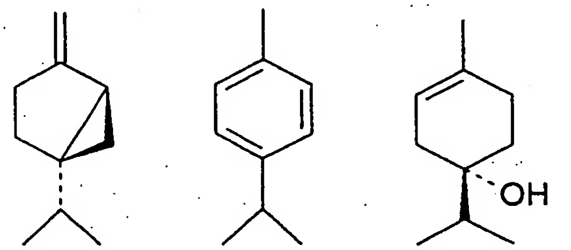
Chewing lice (*Bovicula ovis*) are another major ectoparasite of sheep, which can severely affect the quality of fleeces and hides. Treatment of lice is by similar means to the scab mite, by dipping in solutions containing organophosphates or synthetic pyrethroids. Pour-on treatments based on synthetic pyrethroids have been developed, but in Australia, resistance developed to these products within a few years of their release on to the market (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). The lipophilic nature of the components of the compositions described in the present invention make them ideal for development as pour-on treatments, for which lipophilicity is a key feature.

Sage contains a rich variety of chemicals, many of them terpenes and terpenoids. They include amorphene, aromadendrene, borneol, bornyl acetate, cadinene, camphene, camphor, β-caryophyllene, caryophyllene oxide, 1,8-cineole (eucalyptol), copaene, cubebene, *p*-cymene, geraniol, germacrene D, gurjunene, α-humulene, limonene, linalool, linalyl acetate, manool, myrcene, ocimene, palustrol, phellandrene, α-pinene, β-pinene, sabinene, sabinyl acetate, spathulenol, terpenyl acetate, terpinen-4-ol, terpinene, terpineol, terpinolene, α-thujone, β-thujone, viridifloral. Additional components of sage extracts could include (1R,5R)-epoxysalvial-4(14)-en-1-one, (2R,5E)-epoxycaryophyll-5-en-12-al, (2R,5E)-epoxycaryophyll-5-ene, (2S,5E)-epoxycaryophyll-5-en-12-al, 3-octanol, acetic-acid-ester, α-bisabolol, α-terpineol, α-terpinyl-acetate, arachidic acid, benzaldehyde, β-gurjuene, β-myrcene, β-sitosterol, butyric acid, caprylic acid, cerotinic acid, cis-3-hexen-1-ol, cis-

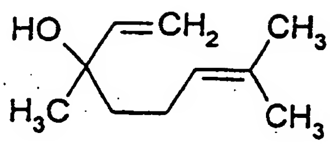
allo-ocimene, cis-beta-ocimene, cis-linalol-oxide, citral, citronellol, cuminaldehyde, delta-3-carene, furfurol, gamma-terpinene, geranial, geraniol, geranyl acetate, isospathulenol, lignoceric acid, linoleic acid, linolenic acid, n-nonanol, n-pentanol, neral, nerol, nerolidol, neryl acetate, oleanolic-acid, oleic-acid, palmitic acid, phellandrene, propionic acid-ester, rosmarinic acid, salvia-4(14)-en-1-one, sclareol, stearic acid, terpinen-4-ol, trans-allo-ocimene, trans-β-ocimene, trans-β-terpineol, trans-linalool oxide, ursolic acid, valeric acid-ester, 5-hydroxy-6,7,4'-trimethoxyflavone, 6,8-di-C-glucosylapigenin, 6-hydroxylutein-6,3'-dimethylether, 6-methoxy apigenin-7-glucoside, 6-methoxy apigenin-7-glucuronide, 6-methoxy luteolin-7-glucoside, 6-methoxy luteolin-7-glucuronide, apigenin-7-glucoside,apigenin-7-glucuronide, betulinic-acid, carnesol, chrysoeriol-7-glucuronide, luteolin-7-glucoside, luteolin-7-glucuronide, luteolin-7-glucuronide-3'-glucoside, luteolin diglucoside, picrosalvin, salvigenin, (E)-nerolidol, 2,6-dimethyl-10-(*p*-tolyl)-undeca-2,6-diene, 2-octanol, 3-octanol, allo-aromadendrene, α-amorphene, α-copaene, α-cubebene, α-gurjunene, α-humulene, α-muurolene, α-phellandrene, α-selinene, β-cyclocitral, cadina-1,4-diene, cis-α-bisabolene, δ-cadinene, γ-muurolene, isoamyl-acetate, isocaryophyllene, isopinocamphone, methyl perillate, myrtenol, myrtenyl acetate, perillaldehyde, perillyl acetate, perillyl alcohol, perillyl-butyrate, t-cadinol, trans-α-bergamotene, trans-calamenene and viridiflorene.

Sage essential oil samples which are most effective in killing parasites usually contain high concentrations of sabinene, p-cymene and/or terpinen-4-ol. Sabinene [(1R/S,5R/S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexane] is a bicyclic monoterpenoid, of the formula $C_{10}H_{16}$, p-cymene [1-

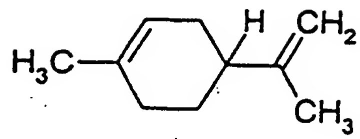
propyl-4-methylbenzene] is a monocyclic monoterpene hydrocarbon, of the formula $C_{10}H_{14}$, and terpinen-4-ol [(S)-1-isopropyl-4-methyl-3-cyclohexen-1-ol] is a cyclic monoterpene alcohol of the formula $C_{10}H_{18}O$, whose respective formulae can be represented thus:



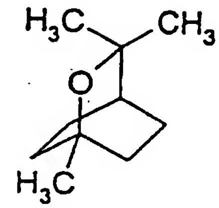
Other compounds tested in the present research include linalool [3,7-dimethyl-1,6-octadien-3-ol], limonene [(R/S)-4-isopropenyl-1-methylcyclohexane], 1,8-cineole [eucalyptol; 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane], camphor [1,7,7-trimethylbicyclo[2.2.1]heptan-2-one], a-terpineol [(R/S)-*p*-menth-1-en-8-ol], a-pinene [(1R/S, 5 R/S)-2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene], b-pinene [(1R/S, 5 R/S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane], borneol [endo-(1R/S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol], myrcene [7-methyl-3-methylen-1,6-octadiene], camphene [(1R/S)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane] and b-caryophyllene [trans-(1R,9S)-8-methylene-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene]. Their formulae can be represented thus:



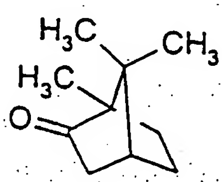
Linalool



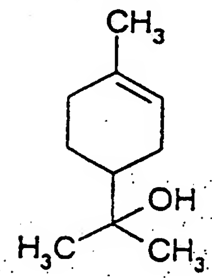
limonene



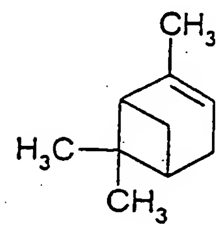
1,8-cineole



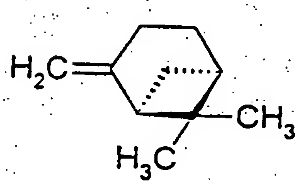
camphor



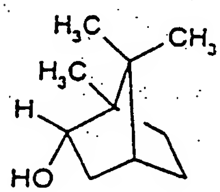
α-terpineol



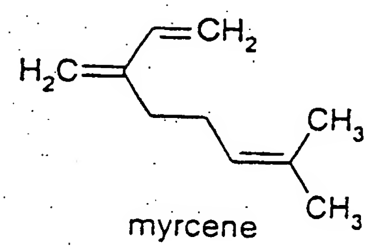
α-pinene



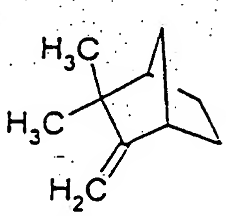
β-pinene



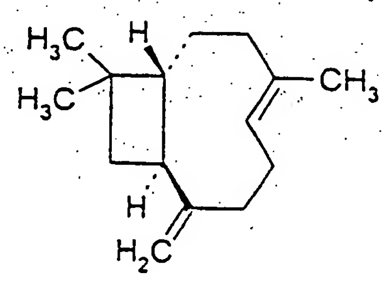
borneol



myrcene



camphene



β-caryophyllene

The parasite-killing activity of these compounds described in the present invention is previously unreported, although they have has been shown to possess other biological activities. For example, US patent 5,635,184 to Camano describes potent antibacterial activity of the essential oil of *Schinus molle*, which contains significant amounts of sabinene. A composition based on volatile monoterpene compounds, one of which could include sabinene, has been described as possessing activity against house- and leopard mites (see Arakawa Chem Ind Ltd (1992) Japanese Patent 0413914A).

It is also desirable for these oils to have low concentrations of thujone and sabinyl acetate, which have been linked to mammalian toxicity (see Tisserand (1995) *Essential Oil Safety- A Guide for Health Care Professionals;* Fournier *et al.* (1993) *Plant Medica* 59: 96-97). Spanish sage (*Salvia lavandulifolia*) is particularly desirable in this regard, due to its low thujone content, and is classified as safe to use, being unlikely to cause toxicity, irritation or sensitisation. Despite the presence of thujone in other Sage essential oils, these have been determined to be of lower toxicity than expected on the basis of their thujone content alone, and are unlikely to cause irritation or sensitisation (see Tisserand (1990) *Essential Oil Safety Data Manual*). The same source also indicates that, for dermal application, essential oils of similar potential toxicity as thujone-rich sage oil could be applied twice weekly at concentrations between 1-2 and 3-5 % by volume. These values are all above the final concentration of sage oil in an aqueous-alcoholic carrier, as described in the present invention.

It is therefore an object of the present invention to provide treatments for parasitic infestations of humans and animals, which are pleasant to use, effective, of low mammalian toxicity, and are unlikely to lead to the development of side-effects or adverse reactions. It is a further object of the invention to provide compositions for treating insect parasitic infestations of fabrics including clothing and furnishings. It is yet a further object of the invention to provide compositions for treatment of parasitic infestations of plants.

In accordance with a first aspect the invention provides a composition comprising an essential oil in a gel carrier, said essential oil being obtained from a plant selected from the genera *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia*, said composition being for use in the treatment of a human or animal having a parasitic insect infestation.

The gel may be based on agar, agarose, gelatin, or a synthetic gelling agent and may be subject to dilution with water and/or alcohol, preferably, isopropyl alcohol. As used herein the term "synthetic gelling agent" refers to a material having the properties of a gel but which is not found in nature.

In accordance with a second aspect the invention provides a composition comprising an essential oil in an aqueous alcoholic vehicle in which the vehicle comprises from about 0.1% up to about 20% alcohol v/v with water and wherein said essential oil is obtained from a plant selected from *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia*, said composition being for use in the

treatment of a human or animal having a parasitic insect infestation.

In accordance with a third aspect the invention provides a composition comprising an essential oil in a vehicle comprising an alcohol/vegetable oil mixture wherein said alcohol is present in said vehicle in an amount of about 0.1% up to about 20% v/v and wherein said essential oil is obtained from a plant selected from the genera *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper or Aloysia*, said composition being for use in the treatment of an animal having a parasitic insect infestation.

In accordance with a fourth aspect the invention provides a composition comprising an essential oil in a gel carrier, said essential oil being obtained from one be more of the following: *Pelargonium, Cymbopagon, Pimpinella, Myrtus (Cretian, Morrocan, orange) Lavandula, Pinus, Melaleucas, Cinnamomum, Apium, Thymus, Hyssopus, Rosmarinus, Cananga, Mentha, Eucalyptus* or *Vitex*.

The properties of the gel carrier may be as already described above.

In accordance with a fifth aspect the invention provides a composition comprising the alkaloid galanthamine for use in the treatment of a human or animal having a parasitic insect infestation. Preferably, the galathamine is comprised in an extract from the plant genus *Narcissus*.

The above described compositions are useful for the killing and/or repelling of parasitic insects in a variety of different contexts. They may be used for

treatment of humans having a parasitic insect infestation, for example head lice or pubic lice. The gel formulations of the first and fourth aspects of the invention are particularly useful for this purpose and may be formulated for topical use such as for hair gels or mousse, for example.

Non-gel formulations such as those described in the second and third aspects of the invention are also suitable for the treatment of humans, for example as a shampoo, but are also well-suited to the treatment of domestic and agricultural animals, as well as insect infested clothing, furnishings and plants. For such applications they may be formululated into sprays, dips or pour-on solutions. Formulations using vegetable oil/alcohol vehicles such as set forth in the third aspect of the invention are particularly suited to the topical treatment of animals suffering from insect infestations.

Further preferred embodiments of the compositions and methods of the invention are disclosed herein below and in the accompanying examples and claims.

As aforesaid various individual components of essential oils of the plant genera described above which are terpenes or terpinoid compounds have been shown to possess insecticidal activity, particularly against lice.

Thus, in accordance with yet a further aspect the invention provides compositions comprising any one of/or combination of, the terpenes and terperoids shown to be insecticidally active according to Figures 7 to 10 of this application for any one of the above described insecticidal uses, whether or not said terpene or terpinoid is comprised in a plant essential

oil or not. Preferred compositions are those comprising sabinene, p-cymene, β-pinene, mycrene, limonene and terpinen-4-ol or compositions comprising combinations of two or more thereof. Particularly preferred are compositions comprising sabinine in combination with limonene and/or terpinen-4-ol.

Also preferred are compositions having concentrations of an insecticially active terpene or terpinoid which is higher than would be found in the essential oil of one of the aforementioned plant genera. For example, the level of terpenes and terpinoids in essential oils may be enhanced by mixing the essential oil with ethanol and partitioning the resulting solution with known volumes of water. Preferred elevated terpene and terpenoid compositions comprise from about 4 to about 25% of the terpene or terpinoid or mixtures thereof. A particularly preferred composition comprises from about 4 to about 25% sabinene.

Compositions comprising terpenes and terpinoids as described above may be formulated in any of the ways described herein and are suitable for any of the various applications described herein.

The present invention is based on the discovery that essential oils can effectively kill insects, especially parasitic insects such as ectoparasites. Certain oils, including those from *Salvia* species and other plant species containing relatively high concentrations of sabinene p-cymene, β-pinene, myrcene or terpinen-4-ol have been found, surprisingly, to be most effective. and therefore of considerable commercial potential. Species of *Salvia* suitable for use in the present invention include *Salvia aethiopis, Salvia amissa, Salvia apiana, Salvia argentea, Salvia arizonica, Salvia azurea, Salvia ballotiflora, Salvia*

blodgettii, *Salvia brandegei*, *Salvia carduacea*, *Salvia carnosa*, *Salvia chapmanii*, *Salvia chia*, *Salvia clevelandii*, *Salvia coccinea*, *Salvia columbariae*, *Salvia davidsonii*, *Salvia divinorum*, *Salvia dolichantha*, *Salvia dorrii*, *Salvia earlei*, *Salvia engelmannii*, *Salvia eremostachya*, *Salvia farinacea*, *Salvia funerea*, *Salvia glutinosa*, *Salvia grahamii*, *Salvia greatae*, *Salvia greggii*, *Salvia henryi*, *Salvia hispanica*, *Salvia lancifolia*, *Salvia lemmonii*, *Salvia leptophylla*, *Salvia leucophylla*, *Salvia longistyla*, *Salvia lycioides*, *Salvia lyrata*, *Salvia mellifera*, *Salvia micrantha*, *Salvia microphylla*, *Salvia misella*, *Salvia mohavensis*, *Salvia munzii*, *Salvia nemorosa*, *Salvia nutans*, *Salvia occidentalis*, *Salvia officinalis*, *Salvia pachyphylla*, *Salvia parryi*, *Salvia penstemonoides*, *Salvia pinguifolia*, *Salvia pitcheri*, *Salvia potus*, *Salvia pratensis*, *Salvia privoides*, *Salvia ramosissima*, *Salvia reflexa*, *Salvia regla*, *Salvia riparia*, *Salvia roemeriana*, *Salvia sclarea*, *Salvia serotina*, *Salvia sonomensis*, *Salvia spathacea*, *Salvia splendens*, *Salvia subincisa*, *Salvia summa*, *Salvia texana*, *Salvia thomasiana*, *Salvia tiliifolia*, *Salvia urticifolia*, *Salvia vaseyi*, *Salvia verbenacea*, *Salvia verticillata*, *Salvia vinacea*, *Salvia virgata*, *Salvia* X *bernardina*, *Salvia* X *palmeri*, *Salvia* X *superba* and *Salvia* X *sylvestris*. Particularly preferred are *Salvia lavandulifolia* or *S. officinalis*. Other species suitable for use in the present invention include *Artemesia dracunculus* (Tarragon), *Citrus*, in particular *Citrus limon* (lemon), *Juniperus communis* (Juniper), *Laurus nobilis* (Bay), *Myristica fragrans* (Nutmeg), *Origanum Vulgare* (Oregano), *Piper cubeba* (Cubebs) and *Aloysia gratissima* (Whitebrush). The oils can be formulated into both aqueous and non-aqueous formulations which remain active against lice. The preferred formulation, in which the oil maintains

significant activity at relatively low concentrations is based on a gel, in which the essential oil is dispersed. This has the advantage over mixed aqueous alcoholic formulations to which sensitive individuals may react adversely due to their alcohol content. In a mixed alcoholic-aqueous carrier, concentrations of oil required to kill human lice are comparable to those of the (organophosphate-based) active components in commercial head lice treatments.

The present invention therefore may be summarised as an effective dose of an essential oil or plant extract, preferably Spanish sage essential oil in the concentration range 0.1-50 % (w/v), in a suitable carrier vehicle based on a carrier oil, such as vegetable oil, an aqueous-alcoholic vehicle, such as IPA-water with IPA in the concentration range 0-50 %, or, preferably in a gel formulation, such as Lubrajel TW (available from United Guardian Inc of 230, Marcus Blvd, Hauppauge, New York, 11788), containing water (0-66 % v/v), IPA (0-20 %) and/or additional plant extracts, as required. For example, the preferred formulation for treatment of human lice is Lubrajel TW diluted 1:2 (v/v) with water, to which 4 % (w/v) Spanish sage essential oil and 0.5 % (w/v) decolourised *Aloe vera* gel are added.

The ability of sabinene-containing essential oils, and in particular those from plants of the genus *Salvia* (sage), to kill insects, and in particular parasitic lice and mites is demonstrated in the examples herein. Essential oils, and in particular the oils from *lavandulifolia* and *S. officinalis* "petite feuille Banon", typically containing high concentrations of sabinene, and low concentrations of thujone, immobilise both head- and clothing lice almost immediately on contact, and remain highly active when

appropriately formulated. As described above the present invention incorporates a number of embodiments comprising a range of formulation types including carrier oils, mixed water-alcohol vehicles and gel forms suitable for topical application to the head or skin of humans, application to furnishings and clothing and to plants. The same preparations are also effective treatments for animal ectoparasites, for example sheep parasites, including *Psoroptes* (mite) and *Bovicula* (louse) species.

It is further demonstrated herein that a number of terpene and terpenoid components of the sage oil synergistically interact, and kill ectoparasites at lower doses than when applied individually in an *in vitro* model. (see Example 4).

The terpene hydrocarbon content of essential oil samples can be elevated by mixing with ethanol, and partitioning the resulting solution with known volumes of water. The most effective ratio of solvents for these experiments is 5 volumes of ethanol:water (3:2) per volume of essential oil, although a range of ratios, from 1:1:1 to 6:4:1 (ethanol:water:essential oil) are also effective. When the partitioning is repeated, the resulting oil shows an increased content of terpene hydrocarbons.

Previously, the lice-repellency activity of *Salvia sclarea* essential oil and terpene or terpenoid components characteristic of this, and related oils, has been described (see Eini *et al.* 1995 US Patent 5,411,992). The lice- and mite-killing activity of sage essential oils, and their chemical components is previously unreported.

Activity of essential oils and terpene or terpenoid components of essential oils was tested using an *in vitro* model system. Test solutions were prepared by accurately dissolving and mixing essential oils or terpenoid components in a range of carrier solutions, including water, isopropanol (propan-2-ol; IPA), water-IPA mixtures in the range 10-90 % by volume IPA and inactive vegetable oils, such as grape seed oil and a range of gel formulations, containing 0-66% water and/or 0-20% IPA. Clothing lice (*Pediculus humanus humanus*), head lice (*Pediculus humanus capitis*) or sheep biting lice (*Bovicula ovis*) were then transferred to small volumes (typically 1-2 ml) of these test solutions, in glass containers, and were left in contact with the solutions for 10 minutes. During this time the solutions were occasionally shaken gently to ensure adequate contact between the lice and the test solution. After 10 minutes, the lice were removed, and placed on filter paper to remove excess treatment.

Exposure of lice to test substances in this way (treatments or controls) resulted in the lice being temporarily immobilised. The lice were then monitored for signs of physical activity for a period of up to 90 minutes. Lice exposed to non-active test substances or controls typically regained activity within 5 minutes of their removal from the test solution; those which failed to regain any activity within a post-treatment period of 30-90 minutes were classified as dead.

Due to their smaller size, parasitic mites (*Psoroptes ovis* or *P. cuniculi*) were treated by adding droplets of test solution to the parasites. The time taken for the mites to stop moving in the test solution was

taken as an indication of the toxicity of the test substance. In all other respects, the experiments were performed similarly to those used to study parasitic lice.

Each experiment was performed at a range of concentrations of the test substance, to determine the dose-response of these materials. Activity of the treatments was expressed as an LD_{50}, which was the concentration of essential oil or terpenoid compound sufficient to kill half of the parasites exposed to that treatment.

In the following non-limiting Examples reference is made to the following figures.

Figure 1 shows the effect of concentration of sage oil in vegetable oil carrier on clothing lice recovery (%);

Figure 2 shows the effect of concentration of sage oil in 20% (v/v) IPA-water on clothing lice recovery (%);

Figure 3 shows data obtained on testing activity on clothing lice of essential oils from a number of plant genera at 20 mg/ml (2% w/v) in an aqueous gel;

Figure 4 shows data obtained on testing activity on clothing lice of essential oils from a number of plant genera at 40 mgl (4% w/v) in an aqueous gel;

Figure 5 shows the effect of sage oil in a vegetable oil carrier against ear mites (*Psoroptes cuniculi*) over a range of sage oil concentrations;

Figure 6 shows the effect of sage oil in a carrier of IPA (20% v/v) in water against ear mites over a range

of sage oil concentrations;

Figure 7 shows effect of a range of terpene and terpenoid components of essential oils in a vegetable oil carrier at 125 - 200 mg/ml against human parasitic lice;

Figure 8 shows the effect of a range of terpene and terpenoid components of essential oils at 25 mg/ml in Lubrajel TW (1 part) and water (2 parts) against human parasitic lice;

Figure 9 shows the effect of a range of terpene and terpenoid components of essential oils at 10 mg/ml in Lubrajel TW (1 part) and water (2 parts) against human parasitic lice; and

Figure 10 shows the effect of a range of terpene and terpinoid components of essential oils in a carrier vegetable oil against *Psoroptes cuniculi*.

Example 1a
Activity of sabinene-containing essential oils against human parasitic lice

At high concentrations, sage essential oil led to gross morphological disruption of the human lice, characterised by abdominal swelling, and the development of an intense red coloration in the body and limbs. When dissolved in an inert (no activity against lice) carrier oil, the LD_{50} for sage essential oil containing as little as 5 % sabinene was in the concentration range of 250-300 $mgml^{-1}$ (Table 1, FIG. 1). In a carrier consisting of IPA (20 % v/v) in water, however, the LD_{50} of the same oil was much lower, at between 3-4 $mgml^{-1}$ (Table 2, FIG. 2). In gel-based formulations, LD_{50} values were below 10 $mgml^{-}$

[1], in the case of gel diluted 1:2 (v/v) with water (Table 3) or with undiluted gel supplemented with 20 % (w/v) IPA. The diluted gel was selected as the preferred formulation, due to the low LD_{50} of sage essential oil it contains, and the absence of alcohol from the formulation, to which certain individuals can be sensitive. Specifically, the preferred formulation is based on Lubrajel TW (a synthetic gel based on glyceryl polymethacrylate and propylene glycol), diluted with deionized water 1:2 (v/v), to which the essential oil, and other components are added, as required.

As head lice (*Pediculus humanus capitis*) were slightly more sensitive to the sage oil formulations than clothing lice (*P. humanus humanus*) (Tables 2 and 4), subsequent experiments were performed using clothing lice as the model system, as concentrations of oils effective against these lice would also be effective against head lice.

Table 1: Effect of concentration of sage oil in vegetable oil carrier on clothing lice recovery (%)

Concentration	Time post-treatment (minutes)					
(mgml^{-1})	0	2	5	10	15	30
0	0	60	80	80	100	100
50	0	40	60	60	60	80
100	0	40	60	60	60	80
150	0	60	60	60	60	60
200	0	40	60	60	60	60
250	0	20	40	40	60	60
300	0	0	0	0	20	20

Table 2: Effect of concentration of sage oil in 20% (v/v) IPA-water on clothing lice recovery (%)

Concentration	Time post-treatment (minutes)					
(mg ml^{-1})	0	2	5	10	15	30
0	0	0	100	100	100	100
1	0	0	100	100	100	100
2	0	33	100	100	100	100
3	0	33	33	33	33	100
4	0	0	33	33	33	33
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	0	0	0	0	0	0

Table 3: Effect of concentration of sage oil in gel diluted 1:2 with water on head lice recovery (%)

Concentration	Time post-treatment (min)				
(mgml^{-1})	0	2	5	10	30
0	0	60	100	100	100
10	0	0	0	0	20
50	0	0	0	0	0
100	0	0	0	0	0

Table 4: Effect of concentration of sage oil in 20% (v/v) IPA-water on head lice recovery (%)

Concentration	Time post-treatment (min)				
(mgml^{-1})	0	2	5	10	30
Control	0	25	50	100	100
1	0	33	67	67	33
2	0	29	43	57	29
3	0	25	50	50	50
4	0	0	0	0	0
5	0	0	0	0	0
10	0	0	0	0	0

Addition of isopropanol (IPA) to vegetable carrier oil, in the range 0-20% (v/v) led to increased activity of the sage oil components, with the IPA itself having no direct effect of the lice over this concentration range. At concentrations of 20% IPA (v/v) in inert (grapeseed) oil, 66-100% of lice treated in this manner recovered activity within 10 minutes. With the addition of a sub-LD_{50} dose (150 $mgml^{-1}$) of sage oil to the grapeseed oil, however, a clear dose response was apparent with increasing concentrations of IPA (Table 5).

Table 5: Effect of concentration of IPA in vegetable oil containing 150 $mgml^{-1}$ sage essential oil on clothing lice recovery (%)

IPA concentration	Time post-treatment (minutes)				
(% v/v)	0	5	10	15	30
5	0	57	71	71	71
10	0	33	50	50	50
15	0	0	0	0	13
20	0	0	0	0	0

In the case of the gel-based formulations, addition of IPA had varying effects, depending on the gel strength. In strong gels (undiluted with water), activity increased with the addition of IPA over the range 0-20 % (v/v), but in weaker gels, (diluted 1:1 or 1:2 with water), addition of IPA over the same range reduced activity (Table 6).

Table 6: Effect of gel strength and IPA content of formulations containing 10 $mgml^{-1}$ sage essential oil on clothing lice recovery after 30 minutes (%)

Gel strength	Lice recovery (%)	
	No IPA	20 IPA (v/v)
Undiluted	100	20
Diluted 1:1 (v/v)	60	60
Diluted 1:2 (v/v)	20	100

A number of essential oil samples were studied for activity, and data from gas chromatographic analysis

of the most active oil samples (*S. officinalis* "petite feuille Banon" and Salvia lavandulifolia), is given in tables 7 and 8.

Table 7: Identified chemical components of the essential oil of *Salvia officinalis* "petite feuille Banon"

Name	Percentage peak area
a-pinene	4.26
camphene	5.61
sabinene/β-pinene	8.03
myrcene	3.16
limonene/1,8-cineole/ *p*-cymene	33.80
linalool	0.32
a-thujone	0.47
b-thujone	0.22
camphor	9.51
borneol/isoborneol	2.29
terpinen-4-ol	1.71
a-terpineol	n.d.
linalyl acetate	n.d.
b-caryophyllene	3.90

n.d. Not determined

Table 8: Identified chemical components of the essential oil of *Salvia lavandulifolia*

Name	Percentage peak area
a-pinene	12.69
camphene	9.39
sabinene/b-pinene	2.34
myrcene	0.97
limonene/1,8-cineole/ *p*-cymene	37.46
linalool	0.78
a-thujone	0.17
b-thujone	n.d.
camphor	22.98
borneol/isoborneol	1.62
terpinen-4-ol	0.15
a-terpineol	1.08
linalyl acetate	1.35
b-caryophyllene	0.94

n.d. Not determined

When incorporated into the preferred formulation, Spanish sage essential oil was more effective as a pediculicide than any of the other essential oils tested, including those known from the prior art. After exposure to sage essential oil at a concentration of 20 mgml^{-1} (2 % w/v), clothing lice started a (very limited) recovery after 50 minutes, later than all the other oil samples tested (FIG. 3).

At 40 mgml^{-1} (4 % w/v), lice showed no recovery when exposed to sage oil, or a number of other oils (FIG. 4), but, due to the greater activity at 2% (w/v), sage oil was clearly the most active oil tested.

A number of oils in addition to sage oils were also tested in the aqueous-alcoholic and vegetable oil carrier systems. At a concentrations of between 2 and 10 mgml^{-1} in the IPA-water carrier, the essential oils from cubebs (*Piper cubeba*) and lemon (*Citrus limon*) were most active, killing 100% and 66% of clothing lice, as assessed 60 minutes after treatment. In the vegetable oil carrier system (at concentrations of 300 mgml^{-1}), nutmeg (*Myristica fragrans*), lemon (*Citrus limon*) and tarragon (*Artemisia dracunculus*) were also all active against at least 66% of the clothing lice treated. Concentrations of *Salvia lavandulifolia* essential oil of as little as 4 mgml^{-1} in 20% IPA-water were sufficient to kill all treated lice in some experiments, compared to 6 mgml^{-1} for the essential oil of tea tree (*Melaleuca alternifolia*), which is commonly used as an "alternative" treatment for head lice, indicating sage oil is more potent than tea-tree oil against these lice.

Example 1b
Activity of sabinene-containing essential oils in alcohol-water and carrier oils against parasites of non-human species

When dissolved in an inert carrier oil, the LD_{50} for sage essential oil containing as little as 5 % sabinene against ear mites (*Psoroptes cuniculi*) was in the concentration range of 50-100 mgml^{-1} (FIG. 5). In

a carrier consisting of IPA (20 % v/v) in water, however, the LD_{50} of the same oil was lower, at between 4-5 $mgml^{-1}$ (FIG. 6). Against biting lice (*Bovicula ovis*), sage oil in an inert oil carrier at 300 $mgml^{-1}$ or in 20% IPA-water at 5 $mgml^{-1}$ was highly effective at killing lice after an exposure time of 10 minutes.

Example 2a

Activity of terpene and terpenoid essential oil components in carrier oil against human parasitic lice.

A number of terpene and terpenoid components of the essential oil of sage are commercially available. A range of these compounds were tested for relative activity by dissolving/mixing the compounds at equimolar concentrations in inert vegetable carrier oil. For the comparative studies, all components were prepared to a concentration of 0.97M (approximately 125-200 $mgml^{-1}$, depending on molecular weight). To avoid only partial solubility in predominantly aqueous carrier media, vegetable oil was selected as the carrier was these studies; all compounds were fully soluble in this medium.

These experiments demonstrated that there was great variation in the activity of the essential oil components against clothing lice, ranging from complete activity (sabinene) through to no detectable activity (1,8-cineole). The other components showed intermediate activity, with limonene and caryophyllene the most active components excluding sabinene (FIG. 7, Table 9). The same compounds were also prepared in a

gel formulation containing Lubrajel TW (1 part) and water (2 parts) at 25 $mgml^{-1}$ (FIG. 8), and those displaying complete activity at this concentration were then tested again at 10 $mgml^{-1}$ (FIG. 9).

Table 9: Effect of sage oil components on recovery (%) of clothing lice
(all compounds at equimolar concentrations in carrier oil)

Compound	Time post-treatment (min)					
	0	2	5	10	15	20
borneol	0	0	50	50	50	50
camphene	0	50	50	50	50	50
camphor	0	0	50	50	50	50
b-caryophyllene	0	0	0	0	25	50
1,8-cineole	0	50	100	100	100	100
p-cymene	0	0	50	50	50	50
limonene	0	0	0	25	25	25
linalool	0	0	50	100	100	100
myrcene	0	0	0	50	50	50
a-pinene	0	50	50	50	50	50
sabinene	0	0	0	0	0	0
terpinen-4-ol	0	0	0	50	50	50
terpineol	0	50	50	50	50	50
Carrier oil	0	0	100	100	100	100

Example 2b
Activity of terpene and terpenoid essential oil components in carrier oil against parasites of non-human species.

Against *Psoroptes cuniculi*, as with the human parasitic lice, there was great variation in the activity of the essential oil components, ranging from complete activity (linalool) through to no detectable activity (caryophyllene). The other components showed intermediate activity, with both limonene and sabinene displaying complete activity within 5 minutes of application to the mites (FIG. 10).

Example 3
Activity of sage oil compared to conventional treatments.

Initial studies indicate that the use of sage oil at 5 $mgml^{-1}$ in 20% IPA-water is more effective in killing lice than synthetic insecticides used in commercial treatments on a weight-weight basis in the same solvent system. For example, clothing lice treated with 5 $mgml^{-1}$ sage oil in 20% IPA-water showed no recovery of activity, whereas lice treated similarly with 5 $mgml^{-1}$ malathion or permethrin in 20% IPA-water showed signs of recovery within 1 hour. The legs and antennae of approximately 80% of malathion-treated lice showed twitching motion 40 minutes after treatment, whilst lice exposed to permethrin were able to move (albeit slowly) within 45 minutes of treatment. In both these cases, all the treated lice were able to undertake locomotion within 48 hours of treatment. No such activity was observed in lice treated with 5 $mgml^{-1}$ sage oil.

Example 4

Interaction between isolated monoterpene essential oil components in carrier oil

To test whether isolated terpene and terpenoid components of sage essential oils were able to interact with each other, and kill lice at concentrations below their LD_{50} values when tested in isolation, sub-lethal concentrations of the compounds listed in tables 7 and 8 were prepared in carrier oil. In practice, the concentrations tested were as follows: myrcene (0.24 M), b-caryophyllene (0.24 M), *p*-cymene (0.25 M), terpinen-4-ol (0.32 M), linalool (0.32 M), sabinene (0.37 M), a-terpineol (0.43 M), 1,8-cineole (0.43 M), limonene (0.48 M) and a-pinene (0.73 M). At these concentrations, clothing lice

rapidly recovered activity, typically within 5 minutes of treatment.

Combinations of sabinene with limonene, sabinene with a-terpineol and limonene with terpinen-4-ol and all demonstrated increased activity against lice, compared to the isolated compounds. In these combinations of compounds, the lice failed to recover activity within 5 minutes, whereas the lice exposed to the isolated compounds at sub-lethal concentrations were fully active by this time.

Against ear mites (*Psoroptes cuniculi*), combinations of sabinene with a-terpineol, linalool with a-terpineol and linalool with *p*-cymene were all more active than their monoterpene components applied to the mites in isolation.

Example 5

Other natural compounds active against lice.

In addition to sabinene-containing essential oils, extracts from the bulbs of plants of the genus *Narcissus* were also determined to display potent activity against lice. Galanthamine, an alkaloid found in such extracts, was determined to be particularly active in this regard, and was completely effective against lice at concentrations of 5 $mgml^{-1}$ in 20 % IPA (v/v) in water. The lice-killing activity of galanthamine was possibly due to its potent ability to inhibit acetylcholinesterase, which was determined using spectrophotometric methods (M. Ryan).

Example 6

Efficacy of a formulated product against parasites *in vivo*.

A formulated product containing 4% (w/v) Spanish sage essential oil and 0.5% *Aloe vera* gel (10x concentrated) in Lubrajel TW (1 part)-water (2 parts) was applied to the hair of a volunteer infested with head lice. After initial assessment of the infestation, 60 ml of the treatment was applied to the scalp, and covered with a shower cap for a period of 20 minutes. The cap was then removed, and the product removed by careful washing. The volunteer's hair was combed with a fine-toothed comb to remove dead lice. The procedure was then repeated after 7 days, to allow any eggs not killed by the initial treatment to be hatch. Following the second application, and after a period of 7 further days, there was no sign of lice infestation in the volunteer.

Example 7

Efficacy of a formulated product against agricultural pests

A formulated product containing 0.5-2.0% (w/v) Spanish sage essential oil in 20% (v/v) IPA-water was applied to aphids, both *in situ* (on the plant on which they were feeding), and under *in vitro* conditions. When sprayed onto the aphids, the formulated product quickly immobilised the aphids, and, 60 minutes after application, they remained inactive. Application of the formulated product, in the form of an aerosol, or as a solution, was 100% effective in killing aphids under these conditions.